The new melanoma

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In the last year there have been two great leaps in the development of therapies against metastatic melanoma. A phase III trial of a new antibody treatment showed an overall survival improvement, and a separate phase I trial of a kinase inhibitor yielded significant tumour load reductions. In June, the New England Journal of Medicine released the results of a 676 patient phase III trial in melanoma showing, for the first time, improvement in the overall survival of metastatic patients upon treatment with a CTLA-4 blocking antibody (Ipilimumab). CTLA-4 down-regulates T-cell activation and is important for effecting cancer cell immune escape. By blocking CTLA-4 with the Ipilimumab antibody, T-cell activation is allowed to progress unhindered. The authors reported that under antibody treatment patient survival was increased by a median of four months [1]. A few months later, the same journal published a phase I trial which had used a carefully designed small-molecule (PLX4032) to treat 32 specifically selected metastatic melanoma patients. PLX4032 acts by selectively inhibiting the mutated form of a kinase (BRAF) involved in driving melanoma cell proliferation. The mutation, which constitutively activates BRAF, is present in more than half of all melanomas. The trial authors found that PLX4032 treatment resulted in significant (sometimes spectacular) reductions in the tumour load of 25 patients [2]. These two trials showcase long-sought breakthroughs in the treatment of metastatic melanoma and end decades of what has been unremitting failure.

Behind these scenes of clinical success a quieter revolution has been brewing. In the last few years, critical developments in our understanding of metastatic melanoma have prompted the rise of two new schools of thought concerning how melanoma may progress from a small primary lesion to a widely dispersed (and lethal) metastatic disease. While the hypotheses these schools champion appear at first glance to be diametrically opposed, there are signs of reconciliation which promise to yield a unified and comprehensive "theory of progression".

Until recently, clinicians and researchers had assumed that after the initial transformation event, in which a normal cell is committed to a malignant fate, the disease progresses by evolving from being weakly metastatic to a dangerously aggressive state. It was thought that during disease progression cancer cells continue to acquire genetic changes which increase their capacity to proliferate, avoid cell death and invade tissues, and thus follow a linear progression of increasing metastatic potential. It was hoped that markers of this progression might also be useful as prognostic tools or even targets for immunotherapy and drug intervention. Unfortunately, genetic changes which reliably correlate with melanoma stage progression have not yet been positively identified.

Not long ago it was recognized by some researchers that the evolutionary model of cancer progression was not sufficient to explain the data coming out of scientific and clinical laboratories. For example, whenever a new marker was associated with disease progression closer examination would reveal that its expression in melanoma tissue is quite heterogeneous. A protein reported to be only expressed in the primary lesion, would haphazardly reappear in metastatic tissues. Findings such as these suggested that cancers were considerably more plastic entities than evolutionary models allow. Indeed, melanoma lesions are particularly heterogeneous in their cellular make-up. While it has long been known that lesions include, in addition to melanoma cells, a variety of other cell types, it is only recently that we have come to appreciate the considerable intra-lesional variation which exists between melanoma cells. Cancer stem cell theory, originating from studies performed on acute my-

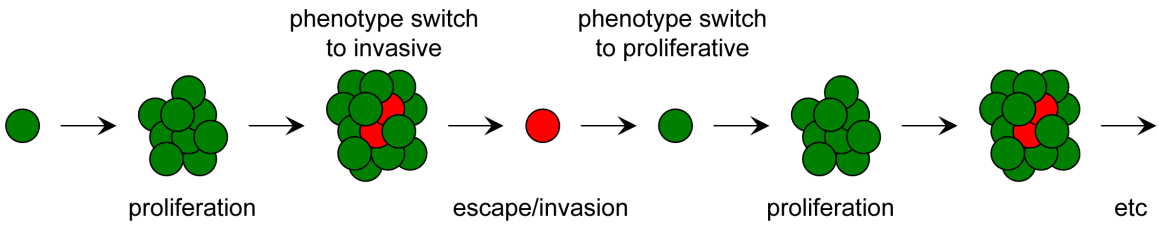

Figure 1 – The phenotype switching model of melanoma progression. The lesion grows via proliferation of proliferative phenotype melanoma cells. Increasing levels of hypoxia inflammation likely contribute to the switching of many cells to an invasive phenotype which then escape the lesion and penetrate distal regions of the body. A small number of invasive phenotype cells are able to switch back to the proliferative phenotype and renew the cycle.

Report

eloid leukemia, proposes that within every lesion there is a tiny population of cells that resemble the stem cells of normal tissues [3]. Cancer stem cells replicate slower than other cancer cells, but can do so indefinitely, and they can give rise to faster replicating daughter cells with different (differentiated) characteristics that make up the bulk of the tumour. These daughter cells are presumed to encounter replicative exhaustion and thus could not provide the seed for other metastases. Therefore it is proposed that a small population of cancer stem cells is wholly responsible for continuous disease progression. This model explains cellular heterogeneity very well, as normal stem cells are known to give rise to differently committed daughter cells. It may well also explain melanoma's persistent resistance to therapy, as cells being targeted may be the bulk-population of fast-cycling daughters, leaving slow-cycling cancer stem cells untouched. To support the notion of a melanoma stem cell, some melanoma researchers have reported the isolation of small, specific populations of cells directly from melanoma tissues which alone retain the tumorigenic activity necessary for driving metastatic progression [4]. But findings such as these have been challenged and the tools used to derive them brought into question. For example, a group injecting randomly selected single melanoma cells into an animal model reported that as much as a quarter of any given lesion's melanoma cell population is tumorigenic [5]. This and other findings have convinced a significant proportion of melanoma researchers that the cancer stem cell model may not be sufficient to explain melanoma biology [6,7].

Other studies of melanoma cell heterogeneity have led to the development of a significantly different model for disease progression. These investigations have mostly been based on studies of melanoma cell cultures. Melanoma cell culturing is, for some clinics, a routine procedure designed to ensure that patient materials may be stored for an indefinite period in a form which is easily analyzable. If, for example, you wish to check for a particular mutation, one need only thaw out and grow sufficient cells in the lab, extract DNA and perform the necessary (and now-a-days trivial) experiments to get the required data. So there are a significant number of sites around the world which maintain stocks of patient-derived melanoma cell cultures. In many of these sites it has been noticed that the expression of a specific collection of several hundred genes can be used to collect culture samples into recognizably distinct groups. Unfortunately, this grouping shows no relationship with patient metrics [8]. These gene expression signatures aren't even patient-specific, as a single patient with multiple lesions can yield melanoma cultures belonging to either group. While this taxonomy of two melanoma cell gene expression signatures was initially mysterious, it was soon discovered that there are important differences in the in vitro behaviours of cell cultures from different groups. The samples of one group express melanocytic markers and proliferate well, but (relative to the other group) are less invasive. The more invasive samples of the other group, however, express few melanocytic markers and often do not proliferate as well as the first group. This and similar data was pointing to the fact that any given melanoma cell culture tended towards having either a proliferative or an invasive phenotype. This raised difficult questions. For example, metastatic potential invokes the capacity for both proliferation and invasion. Why were the cell cultures expressing one or the other of these characteristics? How could proliferative cultures with weakly invasive characteristics nevertheless derive from aggressive and distal metastases? A possible answer emerged when these cells were injected subcutaneously into nude mice (a laboratory animal model without a functioning thymus and thus very few T cells, useful for growing different tissues and cells without fear of rejection). It was found that while there was a significant difference in growth kinetics (proliferative cultures initiated tumourigenesis weeks sooner than invasive cultures) nearly all injected cultures resulted in tumours regardless of phenotype. Critically, immunohistochemical analysis of these xenografts showed the presence of both phenotypes in each. This indicated that either phenotype was capable of being derived from the other, that a form of switching had taken place in some of the cells during tumourigenesis [9]. Phenotype, while a fixed characteristic in vitro, is not fixed in vivo.

These findings have led to the derivation of a strikingly novel hypothesis, termed the phenotype switching model for melanoma progression. The concept is one in which melanoma cells shuttle back-and-forth between states of invasiveness and proliferation in response to changing microenvironmental cues. This model explains why cultures of either phenotype may be obtained at any stage, with each lesion growing to become a patchwork of both. It offers a reason for why the disease is so resistant to standard chemotherapies, which attack rapidly proliferating cells but would have little effect on slowly proliferating invasive phenotypes, cells which can later provide the source for proliferative phenotypes after treatment is halted. Immunotherapies which target melanocytic markers are also compromised by an invasive population which simply does not express them.

There are conspicuous correlations between the phenotype switching model and the idea that melanoma is propagated by stem cells. Cancer stem cells are thought to proliferate slowly, self-renew, provide a reservoir of therapy-resistant cells as well as seed and maintain tumours. All of these are mirrored by the invasive population described by the phenotype switching model. Furthermore, cancer stem cells studies are emerging from melanoma labs which reveal them as being plastic in their expression of defining markers. For example, Meenhard Herlyn's group published a study in which they identify JARID1B as a melanoma stem cell marker, but show that its expression is dynamic and not actually necessary for seeding tumours [10]. One has to wonder, as researchers continue to redefine what passes for a cancer stem cell to the point that even marker expression is not a fixed characteristic, whether or not the differences between invasive phenotype melanoma cells and what are thought to be melanoma stem cells are simply those of semantics? The immediate future holds that the perceived cellular landscape for melanoma is about to undergo a sharp paradigm, as more and more researchers come to accept the shape-shifting nature of these cells, and that the standard cancer stem cell model simply does not

apply to melanoma [7].

Enthusiasm over the recent successes with Ipilimumab and PLX4032 should be tempered by an appreciation of their limits. Ipilimumab grants an average life extension which is shorter than half a year. PLX4032 can effect startling reductions in tumor mass, but there is no evidence that this translates to extended survival. Melanoma is still killing the patients for whom these therapies have a significant effect. Examining therapy response in the context of new cellular hypotheses such as the phenotype switching model of melanoma progression has the potential to extend these gains.

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Dr Hoek obtained his PhD studying the biochemistry of multiple schlerosis at the University of Queensland in Australia. After moving to the USA he began working on gene expression profiling in melanoma, using DNA microarray technology, at Yale University under the supervision of Prof Ruth Halaban. Dr Hoek then transferred to Switzerland, joining Prof Dr Reinhard Dummer's team at the Department of Dermatology, University Hospital of Zurich. He remains strongly interested in studying the underlying molecular biology of melanoma cell heterogeneity.